STUDIES ON Cr(VI) BIOTRANSFORMATION BY LABORATORY GROWN MIXED CULTURE

by

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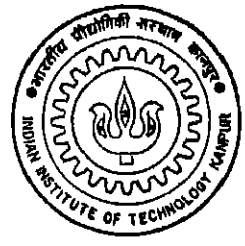

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STUDIES ON Cr(VI) BIOTRANSFORMATION BY LABORATORY GROWN MIXED CULTURE

A thesis submitted
by
B. Giridhar

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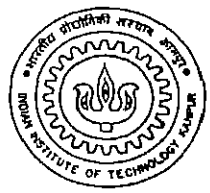

to the
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July 1997

CERTIFICATE

It is certified that the work contained in this thesis entitled **STUDIES ON Cr(VI) BIOTRANSFORMATION BY LABORATORY GROWN MIXED CULTURE**, by B Giridhar, has been carried out under my supervision and that this work has not been submitted elsewhere for a degree

July, 1997

C Venkobachar 24/7/97

Professor
Department of Civil Engineering
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Kanpur.

Dedicated

to
my sisters
vaniyasri and malathi

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ABSTRACT

Hexavalent chromium reduction studies showed that *Bacillus coagulans* possessed the highest Cr(VI) reduction potential among the three bacteria screened Higher initial concentration of *B. coagulans* resulted in a higher Cr(VI) reduction rate. Decrease in the specific growth rate was also observed with increase in Cr(VI) concentration Test biomass, a mixed culture prepared by aerating a mixture of *B. coagulans* and sewage, showed slightly lower reduction rate than pure culture but obviates the use of expensive solid-liquid separation process Test biomass showed a better reduction rate than that of control biomass (absence of inoculated *B. coagulans*) Specific Cr(VI) reduction was similar for both malate and sewage having same COD values as electron donors Further, yeast extract of 6 g/L was required to be added for obatining maximum reduction capacity Cr(VI) biotransformation studies performed in fed batch reactor system with malate as electron donor (for 13 mg/L initial Cr(VI) concentration) showed that both test and control biomass could produce effluents with non-detectable Cr(VI) for an initial Cr(VI) concentration of 13 mg/L When this was increased to 26 and 52 mg/L only test biomass produced effluents with non-detectable Cr(VI) concentration for 6 and 2 d respectively. Test biomass also reduced Cr(VI) when sewage was used as electron donor for an inital Cr(VI) concentration of 26 mg/L These fed batch reactors were operated for more than 20 d

1. INTRODUCTION

Progress of human civilization has always been hinged on the use of metals and its derivatives Infact the ages have been named as Iron, Bronze, Steel, and presently the 'Heavy metal' age. Heavy metals play a significant role in industrial progress and overall prosperity of mankind However, they have also accounted for certain bitter consequences by increasing the risk for human health and the ecosystem. Some of these heavy metals are found to be too hazardous or toxic leading to incidents like *itai itai* and Minamata which are not forgotten

Such catastrophic incidents and the toxicology studies of heavy metals have harbingered in making strict effluent disposal standards Inturn these have been the vanguard for further research to obtain innocuous effluents. Since any such investment by man is furthered by his *quid pro quo* attitude, this research should provide technologies which not only satisfy the effluent standards but also be economically viable.

Development of technologies for treatment of chromium contaminated wastes has similar objectives. Chromium in wastewaters is normally in hexavalent [Cr(VI)] or trivalent form [Cr(III)]. Removal of hexavalent chromium from wastewaters has always proved to be difficult due to its high solubility in the entire pH range of 0-14. Further, compounding to the problem is the high toxicity of hexavalent chromium in comparison to trivalent chromium

Reduction of Cr(VI) to Cr(III), and subsequent hydroxide precipitation of trivalent chromic ion, is the most common method

for hexavalent chromium removal This reduction process involves lowering of the waste stream pH to 2.0-3.0, and convert hexavalent chromium to the trivalent chromium with reducing agents But this reduction is not completely effective, and the amount of residual Cr(VI) depends upon time allowed for reaction, pH of reaction mixture and concentration and type of reducing agent employed. Further, to meet increasingly stringent effluent standards, other methods like adsorption and ion exchange are being explored.

In addition to chemical processes available for Cr(VI) transformation, enzymatic reduction has been reported Certain bacterial species have the ability to convert toxic hexavalent chromium to trivalent form, which possibly represents a useful detoxification process. Although a number of strains of *Pseudomonas* sp , *Bacillus* sp. etc have been identified to posses the capability of transformation, its evaluation for application in technical scale for development of engineered system has been rather limited. Shen and Wang (1995) and Gopalan and Veeramani (1994) have demonstrated the possibility of utilising bacteria for reduction of chromium in sterile conditions.

However, to develop biological processes to treat Cr(VI) containing wastes requires substantial research. Such work should be directed towards evaluating the bacterial reduction in non-sterile conditions, search for a better and inexpensive carbon source, development of devices for higher biomass retention etc. Thus, the main objective of this investigation is to engineer the microbial systems for the biotransformation of Cr(VI) in the treatment of chromium bearing wastewaters.

2. LITERATURE REVIEW

2.1 Scope

With the advent of industrial revolution, there has been an insidious proliferation of waste materials of virtually every kind. Metallic species, one of the most dangerous of these pollutants, cannot be destroyed and hence once released in to the environment tend to accumulate through the food chain posing a serious threat to the ecosystem The occurrence of the episodes like Minamata, consequent of biomagnification of heavy metals through food chain and its impact on human health has resulted in increasingly stringent standards set for their control

Chromium, one of these metallic species, is released into the environment through a large number of industrial operations. Chromium is known for its toxicity to microbes, animals etc. and hence it is necessary to treat discharges containing chromium. This chapter in general deals with the chromium toxicity, treatment options available for wastewaters bearing chromium, microbial transformation of more toxic hexavalent chromium to less toxic trivalent chromium and its application in treatment of effluents containing Cr(VI).

2.2 Chromium

Chromium occurs naturally in earth crust as well as in air, surface and ground water. Normally chromium is found in its hexavalent form or trivalent form in water or wastewater. Cr(VI) is very soluble in water in natural environment and forms divalent oxyanions Cr(VI) (CrO_4^{2-}) or dichromate (CrO_7^{2-}),

depending on the pH of solution whereas Cr(III) is insoluble in water of pH above 7 and precipitates as $Cr(OH)_3$.

Hexavalent chromium compounds have wide spread industrial use in metal pickling and plating operations, in anodizing aluminum, in the leather industry as a tanning agent, in the manufacturing of paints, dyes, explosives, ceramics, paper, stainless-steel manufacturing etc. Trivalent chromium salts are employed as moderants in textile dying, in ceramics and glass industry and in photography Consequently, contamination of the environment by chromium arises form the effluents of such industries U.S EPA estimates that discharges from these industries is as high as 4500 kg per day (Townhill et al., 1978). Further, ground water chromium concentration as high as 2740 mg/L has been reported (OTA, 1984).

2.3 Chromium Toxicity

Chromate toxicity to bacterial (Venitt and Levy, 1974) and animal systems (Roe and Carter, 1969) is well documented. Chromate causes irritation and corrosion of skin and respiratory tract, and is believed to be responsible for lung carcinoma (Bidstrip and Case, 1956). International Agency for Research on Cancer (IARC) has classified Cr(VI) in group 1 (human carcinogen) and Cr(III) in group 3 (The agent, mixture or exposure circumstances is not classifiable as to its carcinogenecity to humans).

Toxic effects of chromium are also felt in the conventional biological treatment systems like activated sludge when present in the wastewaters. Although the effects are lesser if chromium

is in its trivalent form, pronounced effects are observed when present in the form of Cr(VI) (Watson, 1973).

Gocay and Yetis (1991) reported an increase in maximum specific growth rate (μ_m) of bacteria with increase in chromium concentration upto 25 mg/L for the cell residence times (BSRT) less than 8 h. When the BSRT was more than 8 h a significant decrease in biomass concentrations was observed at the same Cr(VI) concentration.

Imai and Gloyna (1990) reported that chromate concentration of 5 mg/L was harmful to a activated sludge system as it resulted in decrease in the amount of biomass in the reactor, thus reducing the efficiency of the system

2.4 Available Technologies for Treatment of Wastewaters Containing Cr(VI)

At present, the most commonly used technology for treatment of heavy metals in wastewaters is chemical precipitation. However, hexavalent chromium, Cr(VI), is highly soluble and cannot be removed by precipitation through the addition of commonly used chemicals such as lime and sodium carbonate (Patterson, 1985). Conventional chemical treatment of wastewater containing Cr(VI) involves reduction of Cr(VI) to Cr(III) by a reducing agent under low pH (2-3) conditions and subsequent adjustment of solution pH to near neutral ranges to precipitate Cr(III) as hydroxides.

Commonly used reducing agents for Cr(VI) reduction are sulphur dioxide (SO_2), sodium bisulphite ($NaHSO_3$), sodium metabisulphite ($Na_2S_2O_5$) and ferrous sulphate ($FeSO_4$). Effluents

from chromium reduction process should be neutralised to the range of zero solubility (pH 8.5-9.0) to minimise the amount of soluble chromium remaining in solution.

Sorbents of biological origin have also been used to extract chromium when present in its trivalent form in the wastewater Cr^{3+} adsorption on to bacterial cell surfaces was reported by Churchill et al (1995) and Tobin et al (1985) Adsorption of Cr(III) to biomass is better at higher pH values 8 to 10.

Ion-exchangers also provide a treatment alternative for removal of chromium from wastewaters. Anion exchangers can be utilised for hexavalent chromium recovery. The advantage with this system is that it allows removal and recovery of hexavalent chromium compounds directly without the involvement of the reduction procedure. This process also produces reusable water and may have an economic edge when water costs are high (Watson, 1973).

2.5 Cr(VI) Removal by Biotransformation

Bacterial transformation found in many metallic minerals such as manganese, metal iron, mercury etc. enable the bacteria to increase their tolerance towards toxicity of heavy metals. One such microbial transformation, which is extensively reported, is reduction of more toxic Cr(VI) to a lesser toxic Cr(III). The earliest report was of *P. dechromaticans* isolated from industrial wastewater, which could use chromate or dichromate as a terminal electron acceptor during anaerobic respiration (Romanenko and Korenkov, 1977). Subsequently many organisms have been identified to posses such capabilities. Cr(VI) has been shown to

serve as the final electron acceptor in respiratory chains of *P. aeruginosa* (Gvozdyak et al., 1986), *Bacillus subtilis* (Gvozdyak et al., 1986), *P. fluorescens* (Bopp and Ehrlich, 1988) and *Enterobacter coloacae* (Wang et al., 1990) in absence of oxygen and nitrate.

While *E. coli* ATCC-33456 could reduce Cr(VI) both in anaerobic and aerobic conditions, the specific reduction capacity as well as rate of reduction was lower in aerobic conditions. Metals such as Cu^{2+} and Zn^{2+} were toxic and resulted in lower Cr(VI) reduction. The presence up to 4000 mg/L of sulphate or 8000 mg/L of nitrate, on the other hand, did not affect the Cr(VI) reduction in anaerobic cultures, indicating that both sulphate and nitrate did not compete with Cr(VI) for electrons (Shen and Wang, 1994). Further, a decrease in anaerobic reduction of chromium(VI) was observed in presence of high concentrations (1000 mg/L) of phenolic compounds.

Microbial reduction of Cr(VI) under both aerobic and anaerobic conditions has also been reported in case of *Pseudomonas fluorescens* LB-300 (Bopp, 1980), however in this case aerobic reduction was much more pronounced. This organism isolated from chromium contaminated sediments of Upper Hudson River, New York, demonstrated plasmid-mediated chromate resistance above 1000 mg $K_2Cr_2O_7$/L. Under anaerobic conditions *P. fluorescens* LB-300 utilised acetate as an electron donor whereas under aerobic conditions the organism used a variety of electron donors for chromate reduction (Bopp and Ehrlich, 1988).

Aerobic reduction of Cr(VI) was also found in case of

Pseudomonas putida (Ishibashi et al., 1990) Gopalan and Veeramani (1994) isolated *Pseudomonas* sp from tannery wastewater which was capable of converting Cr(VI) to Cr(III) in aerobic conditions Glucose was carbon source for the bioconversion. A maximum chromate reduction efficiency of 80 to 90% was observed at a biomass retention period of 60 to 72 hours, with an influent Cr(VI) concentration ranging between 15 to 124 mg/L in a continuously fed reactor. Wang and Xiao (1995) isolated *Bacillus* sp. which could reduce Cr(VI) under aerobic conditions

Ligy et al. (1997) isolated *Bacillus coagulans* from chromium contaminated soils which exhibited high Cr(VI) reduction capacity than *P. aeruginosa* under aerobic conditions. It was found that rate of reduction was higher in aerobic conditions compared to anaerobic conditions. A pH of 7.0 was found to be most suitable for Cr(VI) reduction and no decrease in specific Cr(VI) reduction capacity was found in presence of 1000 mg/L of SO_4^{2-} and NO_3^-. Further, Ligy (1997) demonstrated use of domestic wastewater as potential electron donor for Cr(VI) reduction.

The following are the observations which were made by the researchers irrespective of the species of bacteria involved in reduction of hexavalent chromium.

1) Chromium(VI) reduction rate became slower with continuous decrease of Cr(VI), and finally ceased. This cannot, however, be attributed to the termination of cell metabolism, since even after the maximum capacity of Cr(VI) reduction was reached bacterial cultures continued to

utilise the carbon source or growth of cells was observed (Wang and Shen, 1997)

2) Rate of chromium reduction increased with increase in initial cell density, however the increase was not proportional to the increase in cell density i e specific Cr(VI) reduction rate was higher at lower initial cell concentration (Wang and Shen, 1997, Ligy et al., 1997, DeLeo and Ehrlich, 1994)

3) Mostly aliphatic compounds such as glucose, malate, citrate etc were found to be better electron donors for the bacteria to reduce Cr(VI) However, in few cases aromatic compounds such as benzoate when added as carbon sources could trigger Cr(VI) reduction (Wang and Xiao, 1995)

4) Little accumulation of Cr(VI) or Cr(III) was found in the bacterial biomass as the total chromium in the supernatant was always 70-95% of the Cr(VI) added (Shen and Wang, 1994, Deleo and Ehrlich, 1994; Wang et al., 1989).

2.6 Mechanism of Chromium Biotransformation

Hexavalent chromium compounds are oxidizing agents and chromium during this reaction gets reduced to its trivalent state. The half reaction between $Cr_2O_7^{2-}$ and Cr^{3+} is reported by Sillen and Martel (1964):

$$Cr_2O_7^{2-} + 14\ H^+ + 6e^- = 2\ Cr^{3+} + 7\ H_2O \qquad (2.1)$$

$$E^\circ = 1\ 33\ V$$

It can be seen from equation 2.1 that Cr(VI) transformation is favourable at acidic pH. Since pH on the cell surface is

lower than pH inside the cell biotransformation of Cr(VI) is more favourable on the cell surface (Imai and Glyona, 1990). Further, Shen and Wang (1993) mention that Cr(III) produced from transformation of Cr(VI) inside cell cannot be removed from the cell as long as the cell remains intact. As most of the reduced chromium remains in the solution phase the Cr(VI) reduction can be considered to be largely a surface phenomena and very minor intracellular reduction may be occurring (Shen and Wang, 1993).

Two kinds of enzymatic mechanisms have been proposed for Cr(VI) reduction The aerobic activity of Cr(VI) reduction is generally associated with a soluble protein fraction utilising nicotinamide adinine dinucleotide hydrogenase (NADH) as an electron donor either by necessity or for maximum activity. In most instances, the physiological functions of the electron flow to Cr(VI) through the soluble reductase have not been thoroughly examined. Bacterial respiration can utilise a number of inorganic compounds as terminal acceptors, including O_2, NO_2^-, NO_3^-, SO_4^{2-}, Fe(III) and Mn(IV). Under anaerobic conditions Cr(VI) may also act as a terminal electron acceptor through a membrane-bound reductase activity (Wang et al., 1989, 1990). Studies with *E. cloacae* have implicated the respiratory chain in the transfer of reducing equivalents to Cr(VI) through cytochrome-c (Wang et al., 1991).

Shen and Wang (1993) studied the biochemical characteristics and cellular location of Cr(VI) reductase as well as involvement of the respiratory chain in Cr(VI) reduction activity. In reduction experiments with cell extracts of *E. coli* ATCC-33456

they have found that cell supernatants were able to reduce chromate both in presence and absence of oxygen where as the membrane fraction was unable to reduce Cr(VI). Further, their findings that the H_2-reduced cytochromes (b and d) in membrane fraction were reoxidised by oxygen but not by Cr(VI) while H_2-reduced cytochromes in the cell supernatant fraction were easily reoxidised by both oxygen and Cr(VI) suggested that oxidation of cytochromes by Cr(VI) requires the physical existence of soluble Cr(VI) reductase. They have also reported that though, two mechanisms for Cr(VI) reduction were identified, neither of them was a major energy source of cell metabolism. Hence, Cr(VI) reduction as a cometabolism of cells implies that the organism requires an alternate energy source to maintain its activity. Table 2 1 gives the energy potential available from various pathways of glucose metabolism.

Ligy et al. (1997) experimenting with cell-free extracts of *B. coagulans* in aerobic conditions reported similar results. It was also found that the Cr(VI) reduction did not occur due to the metabolic products of cell and the viability of cell was essential for the transformation to occur. However, cell-free extracts could readily reduce Cr(VI) in absence of external electron donors, but the reduction was higher if NADH was added.

2.7 Application of Chromium(VI) Biotransformation

Although a variety of bacteria have been known to bring about the transformation of Cr(VI) to Cr(III), the application potential of continuous-flow biological processes for such reduction has not been evaluated elaborately. Gopalan and

Table 2.1

Energy Potentially Available from Various Pathways for Glucose Metabolism*

1	$C_6H_{12}O_6 + 6O_2$	⟶	$6\ CO_2 + 6\ H_2O$ ΔG° -121 KJ (Aerobic respiration)
2	$C_6H_{12}O_6 + 2.7\ CrO_4^{2-} + 9.3\ H^+$	⟶	$2.7Cr^{3+} + 2CH_3COO^- + 2HCO_3^- + 6.7\ H_2O$ ΔG° -109 KJ (Partial fermentation Cr(VI) as electron acceptor)
3	$C_6H_{12}O_6 + 0.67\ CrO_4^{2-} + 0.3H^+$	⟶	$0.67\ Cr^{3+} + CH_3COO^- + CH_3CH_2COO^- + HCO_3^- + 1.67\ H_2O$ ΔG° -96 KJ (Fermentation, Cr(VI) as a sink of electrons)
4	$CrO_4^{2-} + 8H^+ + 3$ cytochrome-d (reduced)	⟶	$Cr^{3+} + 4H_2O + 3$ cytochrome-d^+ (oxidized) ΔG° -27 KJ (Cr (VI) as electron acceptor from cytochrome-d)
5	$CrO_4^{2-} + 6.5\ H^+ + 1.5$ NADH	⟶	$Cr^{3+} + 1.5\ NAD^+ + 4\ H_2O$ ΔG° -85 KJ (Cr(VI) as electron acceptor from NADH)

* Adopted from Shen and Wang, 1993

Veeramani (1994) monitored the performance of a continuously fed suspended aerobic reactor with *Pseudomonas* sp. for chromium reduction and reported that sludge retention time was a critical factor regulating chromate reduction efficiency. They observed a maximum chromate reduction efficiency of 80% to 90% at a biomass retention time of 60 to 72 hours, with an influent Cr(VI) concentration ranging from 15 to 124 mg/L However, when the sludge retention time was reduced to 36 hours the efficiency dropped to 40%, with an initial chromium concentration of 34 mg/L. Instances of utilising such chromate reducing bacteria for bioremediation of Cr(VI) contaminated soils have also been reported.

Shen and Wang (1995) developed a two stage bioreactor system for hexavalent chromium removal using pure cultures of *E. coli* The first stage being a cell-growing reactor and a second-stage, a chromium reducing reactor.

Ligy (1997) developed a reactor using *B. coagulans* immobilized in a solid matrix for Cr(VI) reduction studies in a fed batch system. It was found that chromium reduction capacity of the biomass reduced with time and the reactor had to be supplemented with additional biomass for further reduction to take place.

2.8 Summary

In brief, the review of literature shows that options available for treatment of Cr(VI) contaminated wastewater prove to be costly or ineffective to meet the stringent standards (Patterson, 1977). In addition to the existing treatment

options, enzymatic reduction of Cr(VI) has been reported Previously recorded evidence shows that use of biotransformation requires the retention of high concentration of bacterial cells in the reactor. To achieve higher biomass retention various alternatives like 2 stage reactor or immobilisation have been tried. However, work on mixed culture of bacterial strain that avoids maintenance of sterile condition is lacking Further, no work has been reported on utilisation of sewage as source of electron donor for Cr(VI) reduction

3. SCOPE OF THE PRESENT WORK

Biotransformation is widely recognised as an alternative for chromium reduction but little effort has been made to utilise this potential of microorganisms for treatment of wastewaters bearing hexavalent chromium A few reports demonstrated the ability of pure cultures of bacteria for Cr(VI) reduction. Further, retention of high biomass concentrations, high hydraulic retention times and efficient solid-liquid separation are prerequisites to produce good effluent quality. The reduction is dependent on the type of carbon source added. Only costly carbon sources like malate, citrate, glucose, benzoate etc have been used in the transformation of Cr(VI) by earlier investigators (Wang and Xiao, 1995) Thus, for effectively using this biotransformation potential of bacteria for treatment of wastewaters containing Cr(VI), this study proposes to use flocculant mixed culture for better retention of biomass. Further, the effectiveness of domestic wastewater as an inexpensive carbon source for Cr(VI) reduction is also investigated

The study was conducted on the following lines

1. Screening of bacterial isolates for their Cr(VI) reduction potential.
2. Determination of the effect of initial concentrations of chromium on the growth and Cr(VI) reduction potential of bacterial isolates.
3. Development of a mixed culture biomass (designated as test

biomass) having a good Cr(VI) reduction potential.

4 Evaluation of sewage as carbon source (electron donor) for chromium(VI) biotransformation in comparison to malate.

5 Performance of fed batch reactors for Cr(VI) reduction using test biomass.

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Glassware

Glassware used in this present study was manufactured by M/s Borosil Glass Works Ltd. (Bombay, India) It was washed with liquid soap (Rankleen Laboratory Detergent, Ranbaxy, India) followed by washing with tap water and distilled water. Glassware used for analysis of total chromium was thoroughly cleaned with chromic acid and then cleaned with hydrochloric acid so as to make chromium adsorption to be minimum and to remove any remaining traces of chromium. Sterilisation of glassware was done at 180°C for 3 h, whenever necessary, in a hot air oven.

4.1.2 Chemicals

Chemicals used were of analytical grade.

4.1.3 Water

Reagents and test solutions were prepared in fresh distilled water.

4.1.4 Domestic Wastewater

Domestic wastewater used for the experiments was collected from the sump well # 2 near the Student Activity Center of IIT, Kanpur which receives wastewater mainly from residential area. The average composition of wastewater during the study period is given in Table 4.1.

Table 4.1 Average Composition of Domestic Wastewater*

Constituent	Concentration, mg/L Average	Maximum	Minimum
COD (total)	164	240	90
COD (soluble)	75	100	50
BOD_5, 20°C (soluble)	56	75	38
TSS	140	210	40

* source - Umasankar (1997)

4.1.5 Bacterial Cultures

Following bacterial cultures used in the study were obtained from the sources as indicated:

Name	Source
Bacillus circulans	Isolated from virgin soils (Kansal, 1996).
Bacillus coagulans	Isolated from chromium contaminated soil, (Ligy et al., 1996).
Thiaspora pantatropha	Obtained from Center for Environmental Science and Engineering, IIT, Bombay.

4.2 Bacterial Growth Media

The following bacterial growth media were employed in the present study. In all cases pH of the media was adjusted to 7.0 ± 0.1 either with 0.1 N HCl or with 0.1 N NaOH.

M1. [Growth Media] - Bacto tryptone, 10 g; Yeast extract, 5 g; NaCl, 5 g, NaH_2PO_4, 6.7 g and Malate 1 g in one liter of distilled water.

M2· [Cr(VI) Reduction Media] - Yeast extract, 5g, Malate, 0.8 g; NaCl, 0.01 g; NH_4Cl, 0.03 g, K_2HPO_4, 0 03 g, KH_2PO_4, 0.05 g and $MgSO_4$ H_2O, 0.01 g in one liter of distilled water.

M3 [Cr(VI) Reduction Media] - Yeast extract, 5g; NaCl, 0 01 g, NH_4Cl, 0 03 g; K_2HPO_4, 0 03 g, KH_2PO_4, 0.05 g and $MgSO_4.H_2O$, 0 01 g in one liter of domestic wastewater having an average COD 180 mg/L.

For preparation of the solid media for bacterial strains, 23.5 g/L of agar was added to M1 growth media For growth of pure culture and batch experiments the media were sterilised in a pressure cooker at 1 Kg/cm^2 for 15 minutes. Unsterilised media were used in preparation of mixed culture and for reduction experiments in fed batch system. Further, for these experiments concentrated solutions of media were prepared and diluted to obtain the above given concentration.

4.3 Analytical Methods

4.3.1 Hexavalent Chromium

Samples were centrifuged at 4000 g for 15 min. prior to the analysis of hexavalent chromium to remove the bacteria. Hexavalent chromium was determined colorimetrically by reacting the solution containing Cr(VI) with diphenylcarbazide in acidic solutions as described in Standard Methods (1989). The absorbance was measured at 540 nm using a spectrophotometer (Hach DR/3000, USA).

4.3.2 Total Chromium

Centrifuged samples for total chromium analysis were digested with a mixture of sulphuric and nitric acids in

round bottom flasks and then oxidized to Cr(VI) with potassium permanganate before adding diphenylcarbazide for colorimetric determination of hexavalent chromium (Standard Methods, 1989).

4.3.3 Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined by titrimetric method after refluxing the sample with dichromate solution as per Standard Methods (1989).

4.3.4 Biomass Concentrations (Cell Dry Weight)

Optical densities of serial dilutions of cell suspensions were determined by measuring the absorbance at 440 nm using a spectrophotometer and correlated with cell dry weight Cell dry weight was determined by filtering known volume of cell suspension through a previously dried and weighed filter paper of pore size 0.45 μ to obtain total suspended solids (TSS) (Standard Methods, 1989). Since the reduction or growth media consisted of only dissolved solids, the TSS obtained were considered equivalent to the biomass concentrations.

Samples drawn for analysis of cell dry weight were centrifuged and the settled cell mass was washed twice with 0.9% saline water and centrifuged before resuspending in physiological saline water. The optical densities were measured at 440 nm and corresponding cell dry weights were calculated.

4.4 Experimental Methodology

4.4.1 Screening of Bacterial Isolates for Cr(VI) Reduction

4.4.1.1 Growth of Bacteria

From the agar slants *Bacillus circulans*,

Bacillus coagulans and *Thiospora pantatropha* were inoculated separately to 250 mL conical flasks containing 100 mL of sterile bacterial growth media (M1). The flasks were then shaken in an environmental shaker (New Brunswick scientific, USA) at 100 rpm maintained at 30°C for 24 hours. The cells were separated from the media by centrifugation at 2000 g for 20 minutes. The settled cells were washed with previously sterilised physiological solution and again centrifuged before resuspending them in 10 mL of physiological saline solution. 1-2 mL of this suspension was taken for cell dry weight determination.

4.4.1.2 Chromium(VI) Reduction Studies

Batch experiments for Cr(VI) reduction were carried out in 250 mL flasks. The flasks were filled with 100 mL of freshly prepared M2 media for chromium(VI) and sterilised at 1 Kg/cm^2 for 15 minutes. Desired amount of $K_2Cr_2O_7$ stock solution (0.5 M) was added to obtain the required chromium(VI) concentration. To the reaction mixture, known amount of bacterial suspension was added and then shaken in environmental shaker at 100 rpm maintained at 30°C. Samples were withdrawn at predetermined intervals and centrifuged and analysed for residual Cr(VI) concentrations. Reaction blanks (without the addition of bacterial suspension) were similarly prepared and shaken at 100 rpm in the environmental shaker maintained at 30°C.

4.4.2 Effect of Initial Cr(VI) Concentration on the Growth of Bacteria and on Cr(VI) Reduction Rate

B. coagulans cells grown overnight were harvested by centrifuging at 2000 g for 20 min. The cells were washed,

centrifuged and again resuspended in physiological saline solution. 0 5 mL and 1 mL of this suspension were added into different flasks containing sterilised M2 media. Cr(VI) reduction experiment was carried out as described in 4.4 1 2. The samples drawn were analysed for biomass concentration in addition to the residual Cr(VI) concentration

4.4.3 Growth of Mixed Culture

2.5 g (by dry weight) of *B. coagulans* bacterial suspension was inoculated to 1 L of media in a 2 L aspirator bottle To this 0.5 L of domestic wastewater was added to give a mixed status to the bacterial culture. Diffused aeration was provided for 23 h after which the suspension was allowed to settle for 1 h and supernatant was drained out and fresh M1 media was added. This procedure was continued till a well settling granular biomass was developed. This is referred as test biomass.

A mixture of M1 media and domestic wastewater was aerated in a similar manner to obtain the control biomass (without inoculating *B. coagulans*).

4.4.4 Kinetics of Cr(VI) Reduction by Pure Culture and Test Biomass

Bacterial suspension of *B. coagulans* was prepared as described in 4.4.1.1 and added to the sterilised M2 media. Previously prepared test biomass was also added to separate conical flasks containing sterile M2 media. Then the Cr(VI) reduction studies were conducted as described in 4.4.1.2.

4.4.5 Kinetics of Cr(VI) Reduction by Test and Control Biomass

Equal quantity of test and control biomass were added separately to the conical flasks containing M2 media and Cr(VI) biotransformation studies were conducted as described in 4.4 1.2.

4.4.6 Chromium Reduction with Different Electron Donors

Malate and sewage were the two different electron donors tested Malate is the electron donor if M2 media is used while in case of M3 media sewage was the electron donor. After inoculation of test biomass in M2 and M3 media, Cr(VI) reduction experiments were performed as discussed in 4.4 1.2.

A batch experiment was also conducted to compare the Cr(VI) reduction with media containing malate and domestic wastewater of equal COD. For this M2 media containing reduced malate concentration (0.15 g/L) and M3 media were used. Test biomass in equal concentrations was added to these media (sterilised) and then chromate reduction experiments were conducted as in 4.4 1.2.

4.4.7 Chromium Reduction with Different Concentrations of Yeast Extract

To study the effect of different concentrations of yeast extract on Cr(VI) reduction, sterile M3 media was used. But, instead of 5 g/L of yeast extract in M3 media, different concentrations of yeast extract were added and reduction experiment was conducted as described in 4.4.1.2.

4.4.8 Performance of Fed Batch Reactors

About 75 mL each of the test and control biomass

was taken in two separate 2 L aspiratory bottles and made up to 1 L by addition of M2 reduction media $K_2Cr_2O_7$ stock solution (0.5M) was then added to both the solutions to give initial Cr(VI) concentration of 26 mg/L. The contents were aerated for 23 h.

After aeration the reaction mixture was allowed to settle for 1 h and the supernatant was drawn for analysis of Cr(VI) concentration and biomass concentration. Fresh M2 media along with Cr(VI) was added to the settled biomass and aeration was restarted. This was continued till sufficient settling of biomass was observed after 1 h of settling time. The experiments were conducted with 13 and 52 mg/L Cr(VI) concentrations. Similar experiments were also conducted using M3 media with an initial Cr(VI) concentration of 26 mg/L.

5. RESULTS AND DISCUSSION

Biotransformation of more toxic forms of heavy metals to less toxic forms exhibited by certain microbes can be employed for bioremediation of contaminated soils as well as water bodies. Investigation was carried out to evaluate the potential of various microbes for reduction of more toxic hexavalent chromium to less toxic and less soluble trivalent chromium. The bacteria which exhibited maximum reduction potential was grown in a mixed culture so as to improve their flocculating nature so that the costly solid-liquid separation process can be eliminated. Factors affecting the Cr(VI) reduction in both pure and mixed cultures, use of domestic wastewater as a electron donor for Cr(VI) reduction and application of the flocculant sludge for Cr(VI) reduction are presented in this chapter along with pertinent discussion.

5.1 Screening of Microbes for Chromium Reduction

Bacillus circulans, a bacterial isolate from virgin soil, *Thiaspora pantatropha* a filamentous microbe capable of denitrification obtained from Centre for Environmental Science and Engineering (CESE), IIT Bombay, and *Bacillus coagulans* isolated from chromium contaminated soils were screened for their chromium reduction potential. Malate was used as the organic carbon source as it was reported to be a better electron donor than glucose (Ligy et al., 1997). The results of Cr(VI) reduction by these bacterial species under aerobic conditions are presented in Table 5.1. It is evident that the bacterial strain,

B. coagulans, isolated form the site of disposal of chromium electroplating effluents, gave the maximum reduction capacity. In all further experiments *B. coagulans* was employed.

Table.5.1

Screening of Bacteria for Chromium(VI) Reduction

Bacterial strain	Source	Specific Cr(VI) reduction capacity (mg of Cr/g biomass)
1) *B circulans*	Isolate from virgin soil	15
2) *T. pantatropha*	Obtained from CESE, IIT Bombay	36
3) *B coagulans*	Isolate from chromium contaminated soil	114

* mg of Cr(VI) reduced in 24 h per gram of initial biomass added

Initial Biomass conc.: 400 mg/L

Initial Cr(VI) conc.: 52 mg/L

Electron Donor - Malate: 800 mg/L

5.2 Effect of Initial Cr(VI) Concentrations on the Growth of Bacteria and Cr(VI) Reduction Rate

B. coagulans which gave the maximum Cr(VI) reduction was used in further work. Since chromium is considered to be toxic, experiments were conducted to determine effect of chromium concentrations on the growth and Cr(VI) reduction rate of *B. coagulans*, with two initial concentrations of biomass and three initial concentrations of Cr(VI). The bacterial growth curves

are presented in Figure 5.1 Irrespective of Cr(VI) concentration, the growth was more for higher initial biomass concentration, though the biomass concentration reduced with the increasing Cr(VI) concentration. In the absence of Cr(VI) maximum growth of biomass occurred, as expected The specific growth rates were calculated by plotting a semi-log graph between biomass concentration (mg/L) vs time (h) The slopes of the best fit lines gave the specific growth rates (μ) which are presented in Table 5.2. It is evident from the data that the growth rate of *B. coagulans* decreased with increase in initial Cr(VI) concentration.

Table. 5.2

Effect of Cr(VI) on Specific Growth Rate of *B. coagulans*

Chromium(VI) Conc. mg/L	Specific Growth rate (μ) h^{-1}
0	0.51
13	0.45
26	0.41
52	0.37

The residual Cr(VI) concentrations for different initial Cr(VI) concentrations when exposed to two different biomass concentrations are presented in Figure 5.2 For an initial Cr(VI) concentration of 13 mg/L, the residual Cr(VI) concentration reached an undetectable limit in 8 h time, however, it took around 16 h for 26 mg/L of Cr(VI). The centrifuged

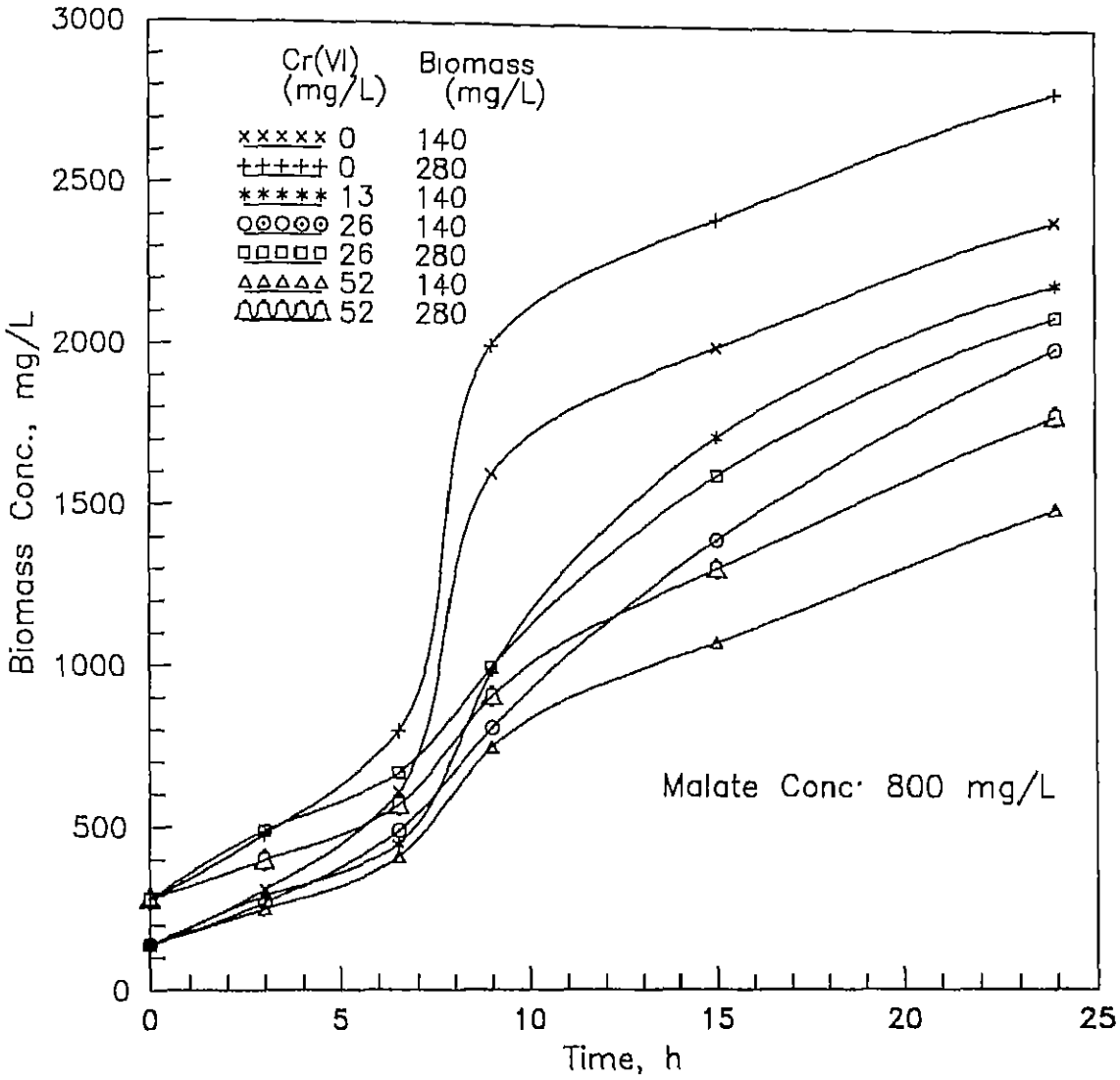


Fig. 5.1 Effect of Cr(VI) Concentration on Growth of *B. coagulans*

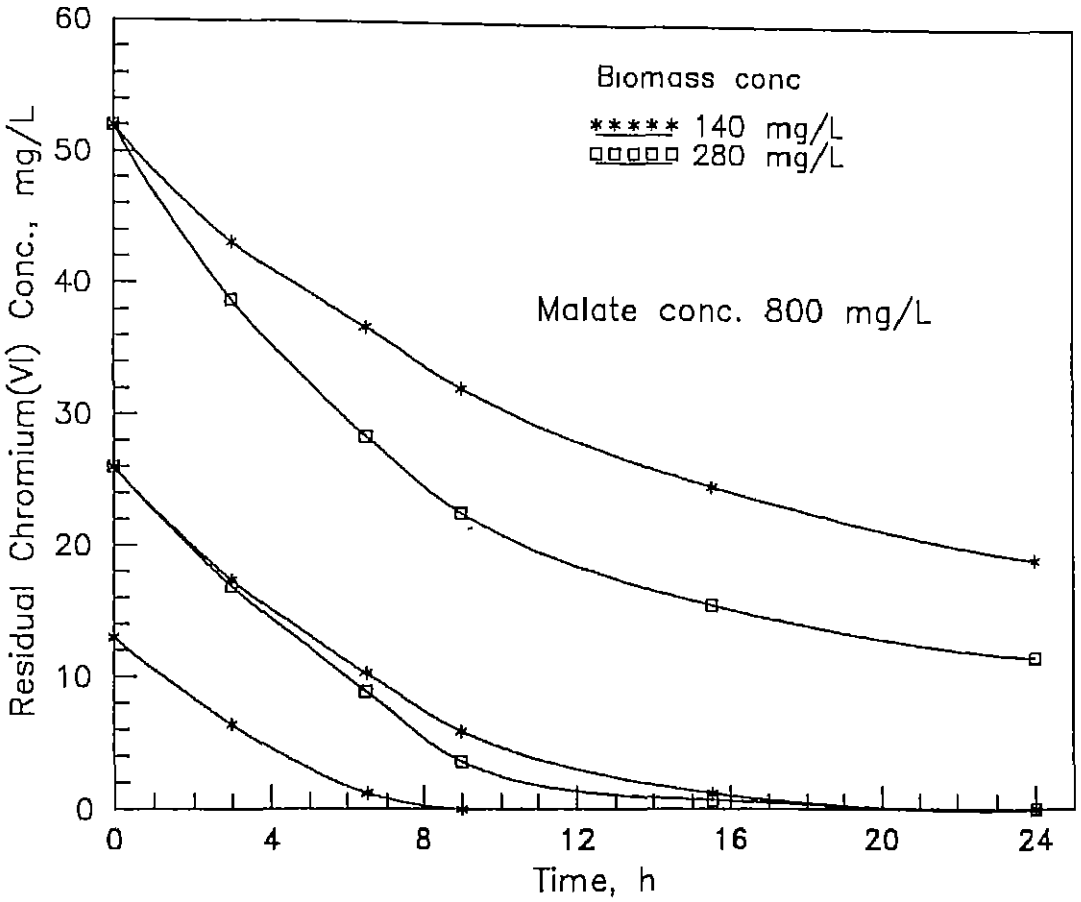


Fig. 5.2 Effect of Initial Biomass Concentration on Cr(VI) Reduction

supernatant when analysed for total chromium consisted of 90-95% of the initial chromium added, confirming the fact that the Cr(VI) was biotransformed to Cr(III). There was no effect of initial biomass concentration on rate of reduction in case of 26 mg/L. But corresponding to a Cr(VI) concentration of 52 mg/L, the chromium reduction had increased with increase in the initial biomass concentration. However, the rate of increase was not proportional to the increase in the amount of initial biomass Such observations were also made with *E. coli* (Shen and Wang, 1994) and *P. fluorescence* LB-300 (DeLeo and Ehrlich, 1994). Further, it was also reported by Horitsu et al (1987) that despite the increase in cell concentration of *P. ambigua* by ten fold the rate of Cr(VI) reduction in the culture did not increase accordingly.

5.3 Mixed Culture

The objective of these studies is to successfully design a process to be used for the treatment of Cr(VI) containing wastewaters. Shen and Wang (1995) and Gopalan and Veeramani (1994) have developed reactors for Cr(VI) reduction using pure cultures of *E. coli* ATCC-33456 and *Pseudomonas* sp. respectively. But maintaining such pure cultures would not only be difficult but also nearly impossible in field conditions. Further, these pure culture bacteria being non-flocculant in nature have a poor settling property and require a energy intensive solid-liquid separation process. In order to obviate this difficulty investigators have utilised immobilised bioreactors (Ligy, 1997) or a two stage reactor system in which the growth of bacteria is

encouraged in the first stage and reduction occurs in the second (Shen and Wang, 1995).

Hence, it was contemplated to develop a biomass of mixed culture which has a good settling property besides having the desirable Cr(VI) reduction capacity. Such a culture was developed by inoculating *B. coagulans* in domestic wastewater containing growth media (M1). After 15 d, a good settleable biomass was developed. This was designated as test biomass as it comprised of *B. coagulans*. A control biomass was also developed in the same manner from wastewater in the absence of inoculated *B. coagulans*. The reduction properties of these biomass were evaluated and compared with that of pure culture of *B. coagulans*.

5.3.1 Kinetics of Cr(VI) Reduction by Pure and Mixed Culture of *B. coagulans*

The purpose of developing a mixed culture of *B. coagulans* for Cr(VI) reduction is to eliminate the difficulties associated with employing the pure culture of *B. coagulans* in practice asides the possibility of developing a biomass with good settling property which can obviate the energy intensive solid-liquid separation process.

The kinetics of Cr(VI) reduction by pure culture of *B. coagulans* and mixed culture of *B. coagulans* (test biomass) is depicted in Figure 5.3 The bacterial cells of density 650 mg/L were subjected to two initial concentrations of Cr(VI) employing malate (800 mg/L) as electron donor. It can be observed that, it takes about 12 h for pure culture of *B. coagulans* and 15 h for test biomass to produce the effluent with Cr(VI) concentration of

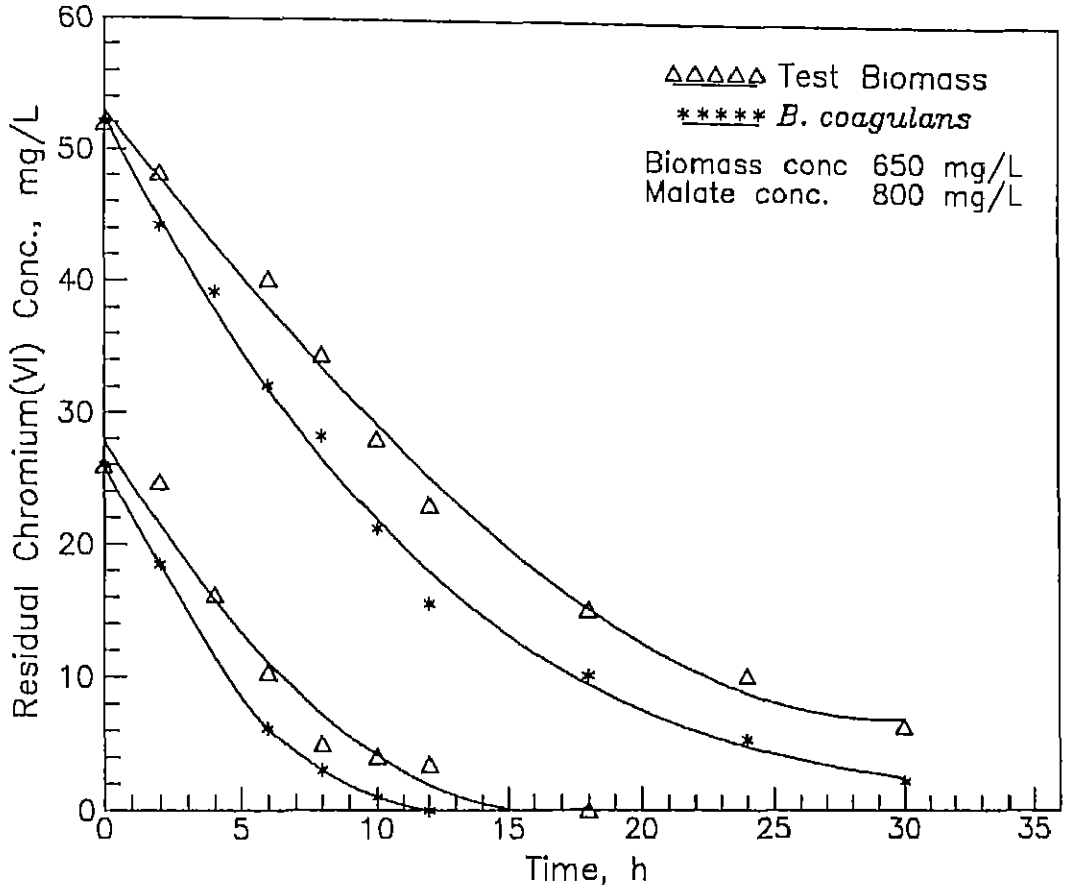


Fig. 5.3 Cr(VI) Reduction by Test Biomass and Pure Culture of *B. coagulans*

undetectable limits for an initial Cr(VI) concentration of 26 mg/L. The Cr(VI) removed had accumulated as Cr(III) in the liquid phase When the two biomass were subjected to higher Cr(VI) concentration, the removal also decreased though pure culture exhibited a higher rate of Cr(VI) reduction than test biomass Employing the mixed culture, though yields slower reduction rate, eliminates centrifugation required for separation of cells prior to effluent discharge.

5.3.2 Kinetics of Cr(VI) Reduction by Test and Control Biomass

Two types of biomass with good settling properties were developed, one in the presence of *B. coagulans* (test biomass) and another in absence of inoculated *B. coagulans* serving as control. The kinetics of Cr(VI) reduction using these biomass with three different influent Cr(VI) concentrations are presented in Figure 5.4. It is clearly evident that the control biomass was less efficient in effecting the Cr(VI) reduction when initial Cr(VI) concentrations of 26 and 52 mg/L were employed It was possible to obtain 100% Cr(VI) reduction within 16 h for 26 mg/L and a longer time for 52 mg/L initial Cr(VI) concentration in case of test biomass, while the control biomass could not effect this even after a long contact time.

5.3.3 Kinetics of Cr(VI) Reduction in Presence of Different Electron Donors

Cr(VI) acts as an effective electron acceptor even in the presence of molecular oxygen to accept electrons generated during the catabolism of malate. It was contemplated whether

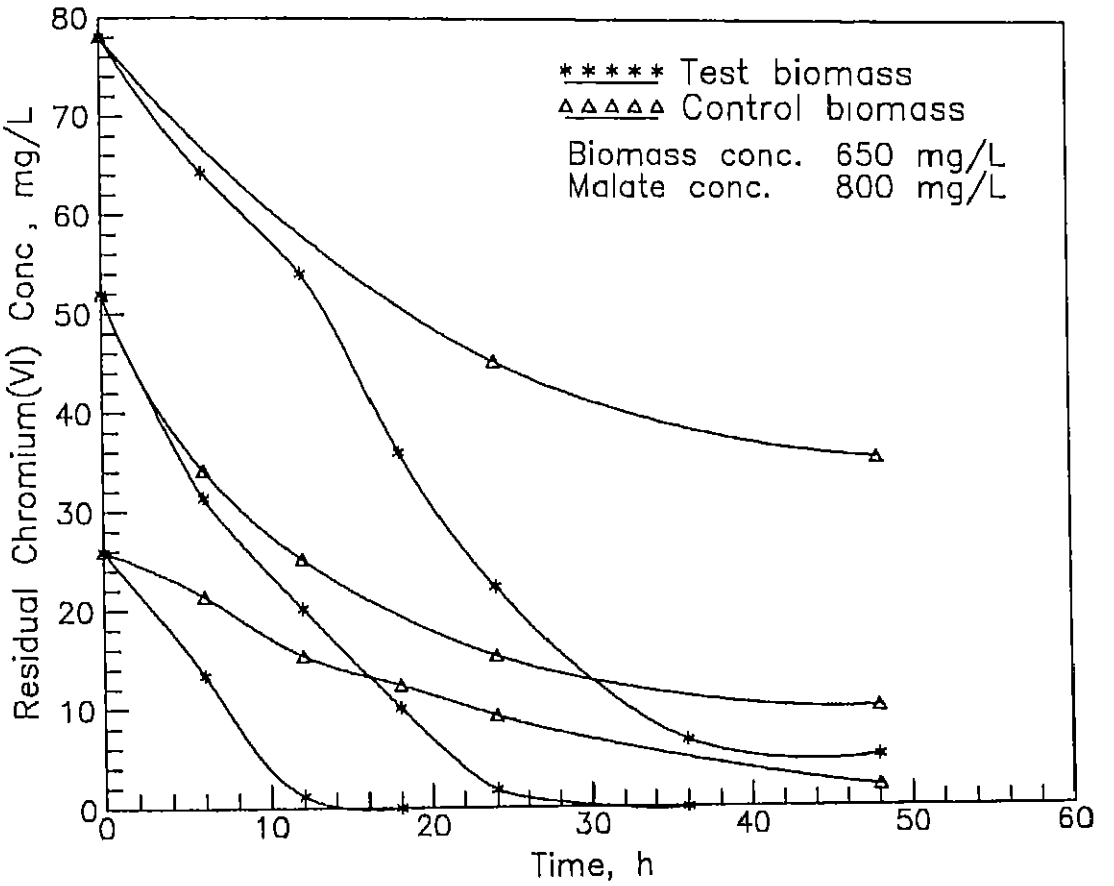


Fig. 5.4 Cr(VI) Reduction by Test and Control Biomass

domestic wastewater can replace the costly electron donor, malate.

Cr(VI) reduction experiments were conducted using test biomass using malate (COD, 955 mg/L) and domestic wastewater (COD, 180 mg/L) as electron donors and the results are presented in Figure 5.5. It can be seen that there is a decrease in Cr(VI) reduction mediated by microbes when sewage was employed in the place of malate, though the reduction was very less. Despite significant difference in COD values of malate and sewage, this decrease in Cr(VI) reduction capacity can be considered as insignificant.

Experiments conducted with lower concentrations of malate (180 mg/L) (Figure 5.6) showed that the rate of reduction was very much similar to that of domestic wastewater of COD 180 mg/L This proves the effectiveness of utilising sewage as electron donor which can prove to be economically more viable alternative than utilising the expensive malate as an electron donor. Further, domestic wastewater having a higher COD may prove to be much more effective than the low strength domestic wastewater of IIT, Kanpur used in this experiment.

As sewage exhibited good Cr(VI) reduction potential, further experiments were conducted using sewage as electron donor with the test biomass.

5.3.4 Effect of Yeast Extract on Cr(VI) Reduction

It is reported in literature that yeast extract is essential for the bacterial biotransformation of Cr(VI) to Cr(III) (Bopp, 1980). Ligy et al. (1997) working with pure

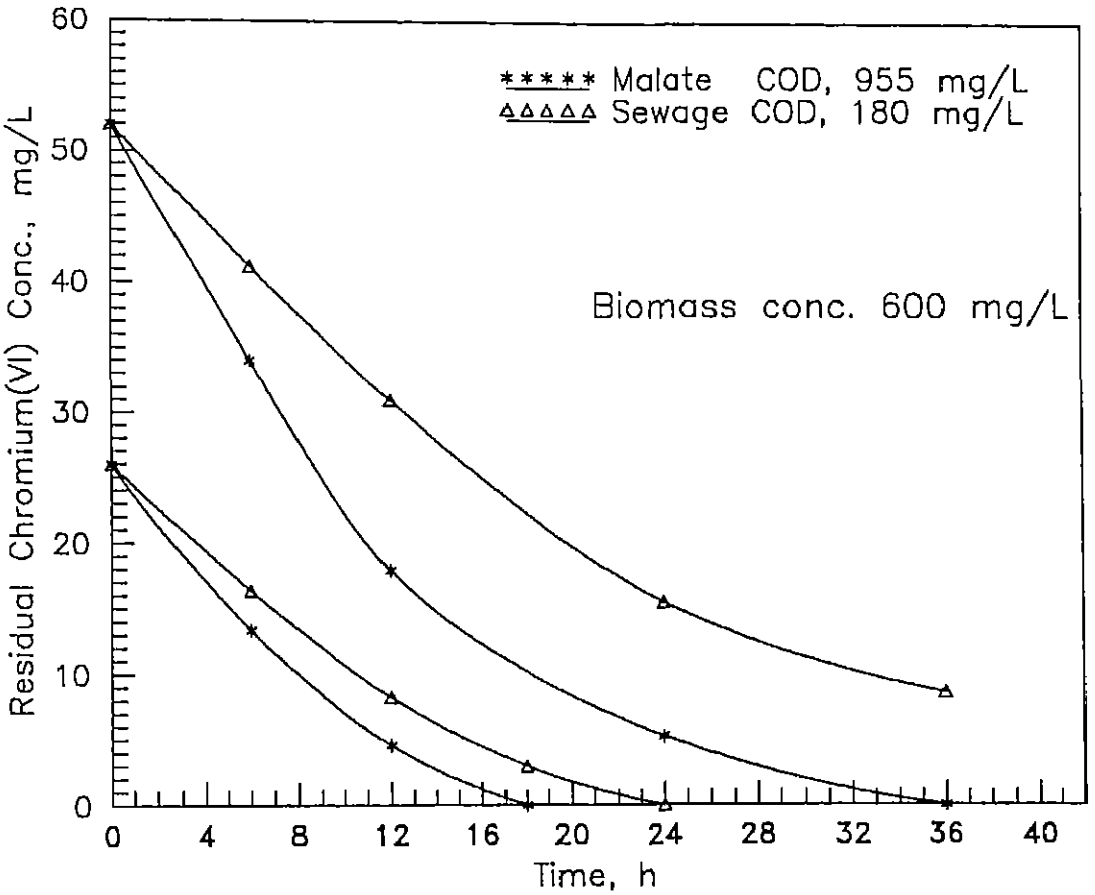


Fig. 5.5 Effect of Malate and Sewage as Electron Donors on Cr(VI) Reduction by Test Biomass

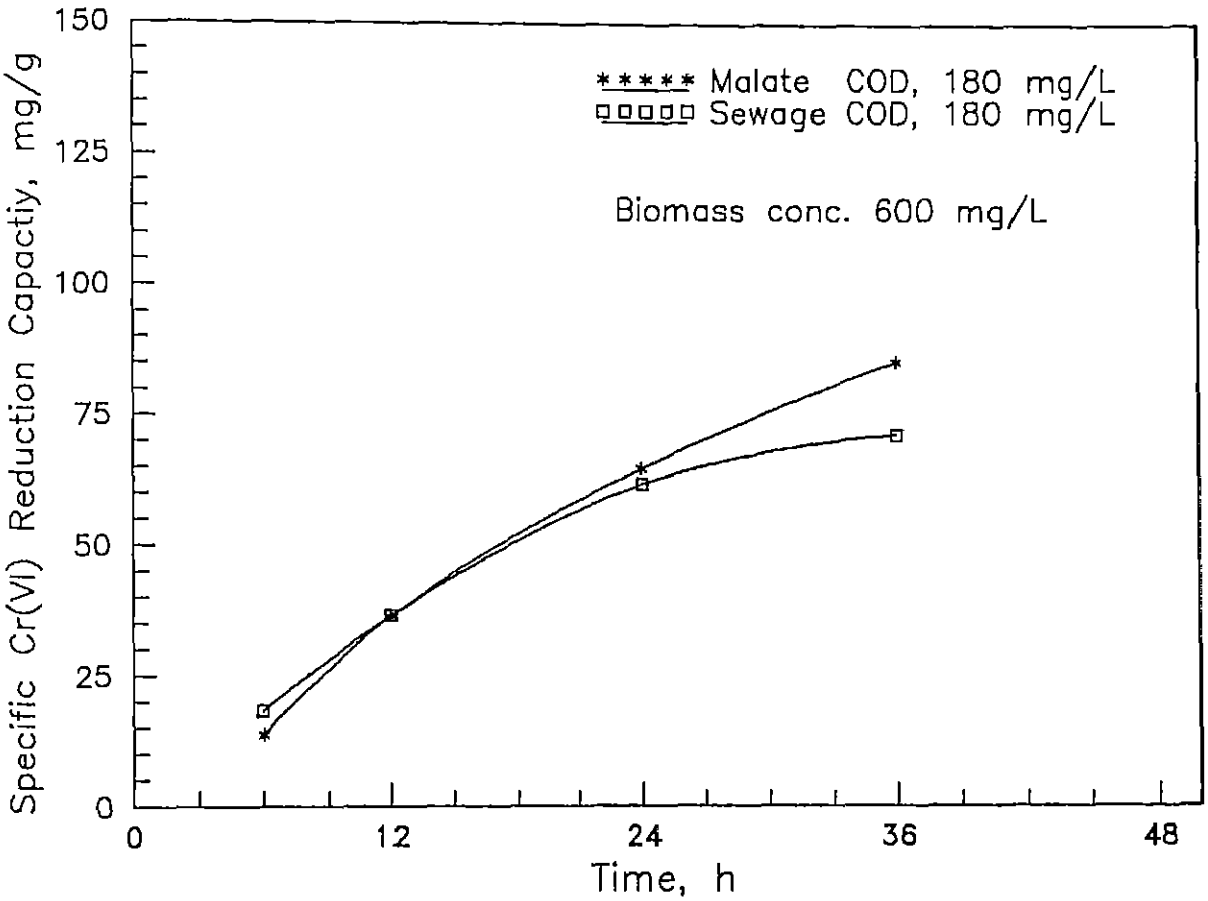


Fig. 5.6 Effect of Malate and Sewage (Equal COD) as Electron Donors on Cr(VI) Reduction by Test Biomass

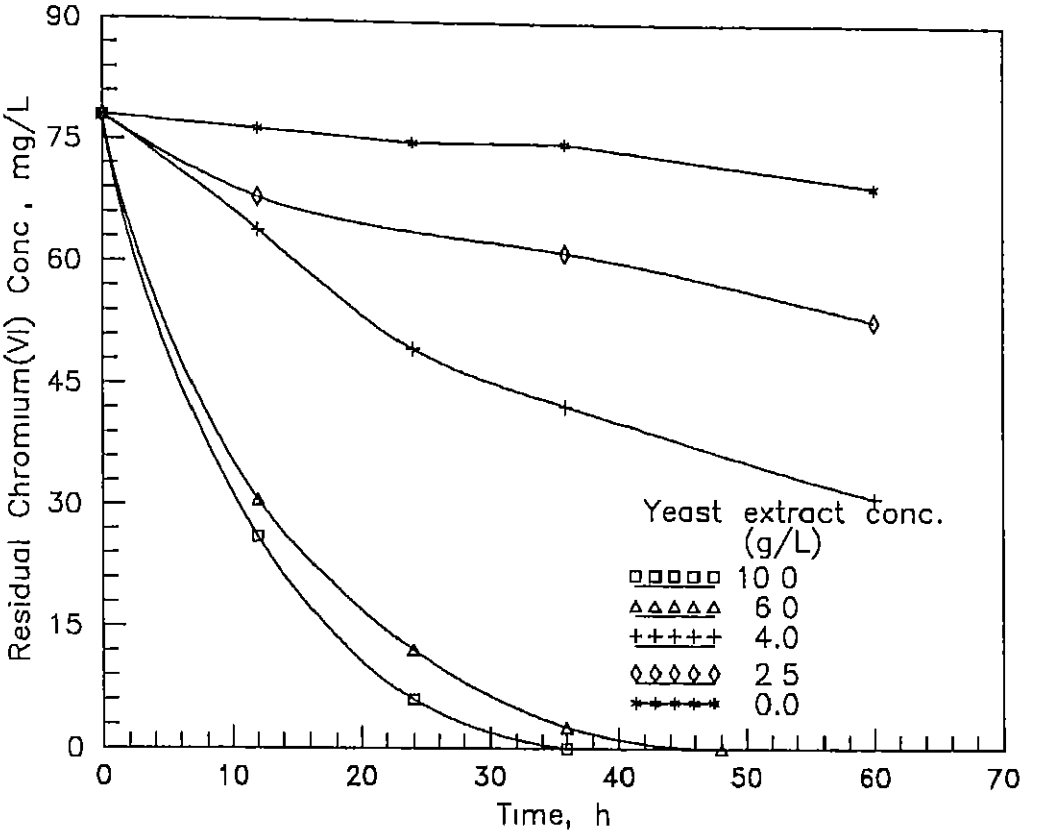
culture of *B. coagulans* found that incorporation of 5 g/L of yeast extract in nutrient media along with malate as electron donor yielded maximum Cr(VI) reduction.

In order to determine the effect of yeast extract on biotransformation of Cr(VI) by mixed culture of *B. coagulans* utilising sewage as carbon source, experiments were conducted in the presence of different concentrations of yeast extract and the results are presented in Figure 5.7. Incorporation of 6 and 10 g/L of yeast extract in the reduction media (M3) yielded 100% reduction of Cr(VI) irrespective of whether 52 or 72 mg/L of initial Cr(VI) concentration was employed. For the lower concentration of yeast extract, the reduction was lesser and it was almost insignificant when yeast extract was absent. The yeast extract may contain undefined components that appear to aid in Cr(VI) reduction. However, yeast extract alone could not trigger Cr(VI) reduction (Ligy et al., 1997).

5.4 Fed Batch System

In the preceding sections, batch experiments on the kinetics of Cr(VI) removal, which is a measure of Cr(VI) reduction potential, yielded valuable information on the utility of mixed culture of bacteria with the dominance of *B. coagulans* isolated from chromium contaminated soils. In this section, efforts are directed to employ the test and control biomass in a fed batch reactor system for Cr(VI) reduction.

Fed batch system operations were conducted in 2 L aspiratory bottles with a provision of aeration for ensuring better contact between microbial biomass and Cr(VI) solution. malate (COD,



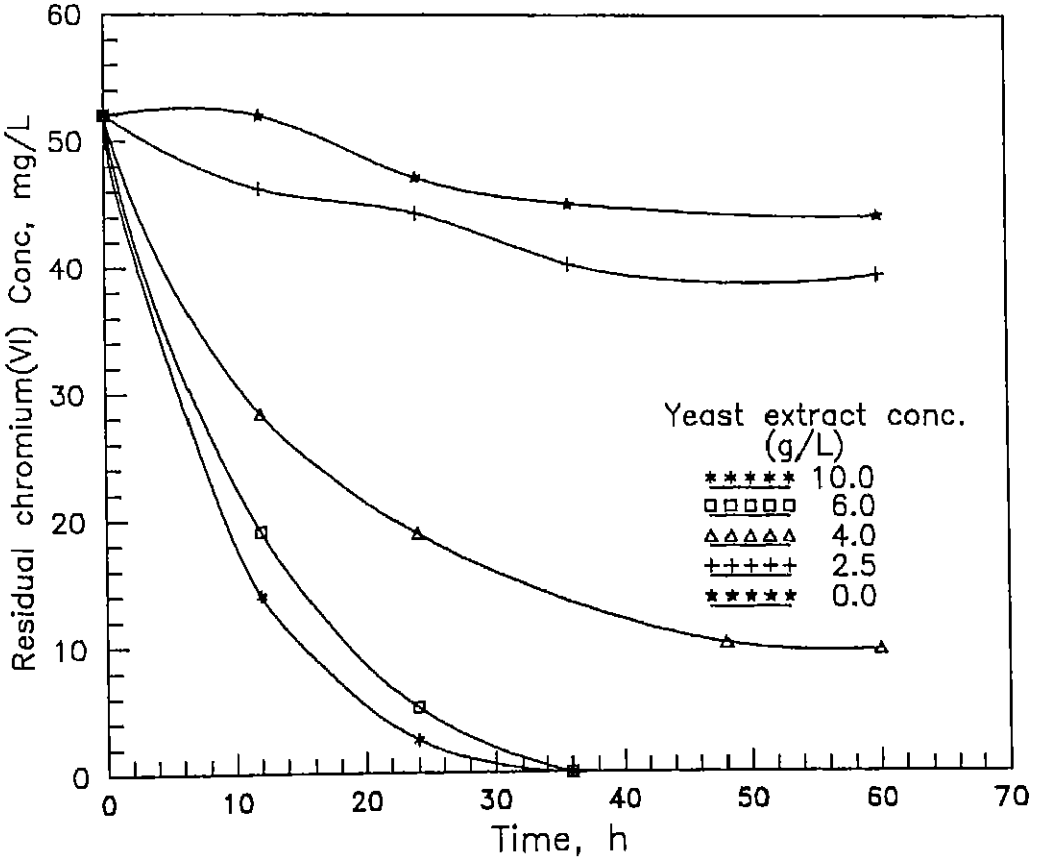


Fig. 5.7 Effect of Yeast Extract on Cr(VI) Reduction by Test Biomass Using Sewage as Electron Donor

955 mg/L) and domestic wastewater (COD, 180 mg/L) were used as electron donors The nutrients along with 6 g/L yeast extract were also incorporated in the Cr(VI) reduction media (M2 & M3) Two batch reactors were operated one having test biomass and another with control biomass. After 23 h of contact, the aeration was stopped and the biomass was allowed to settle for 1 h and the supernatant was centrifuged and analysed for Cr(VI). The biomass was again charged with fresh media containing Cr(VI) to continue the reduction.

5.4.1 Cr(VI) Reduction in Presence of Malate as Electron Donor

In the first set of experiments, two different biomass were exposed to 13 mg/L of Cr(VI) and results are presented in Figure. 5 8 It is evident that both the biomass produced effluents with undetectable Cr(VI) concentration in the effluent over a period of 10 d. Even, the control biomass developed from sewage seems to posses a potential to reduce Cr(VI).

In order to assess the capability of biomass to respond to higher initial Cr(VI) concentration, experiments were conducted with 26 and 52 mg/L. The results presented in Figure 5.9 indicate that the test biomass can handle higher Cr(VI) concentration better than control biomass. Upto 6 d, the test biomass effected 100% reduction of Cr(VI) for 26 mg/L while this did not happen with control biomass, and the effluent Cr(VI) concentration was always around 15 mg/L. Similar results were also obtained with 52 mg/L (Figure 5.10) Even at this high

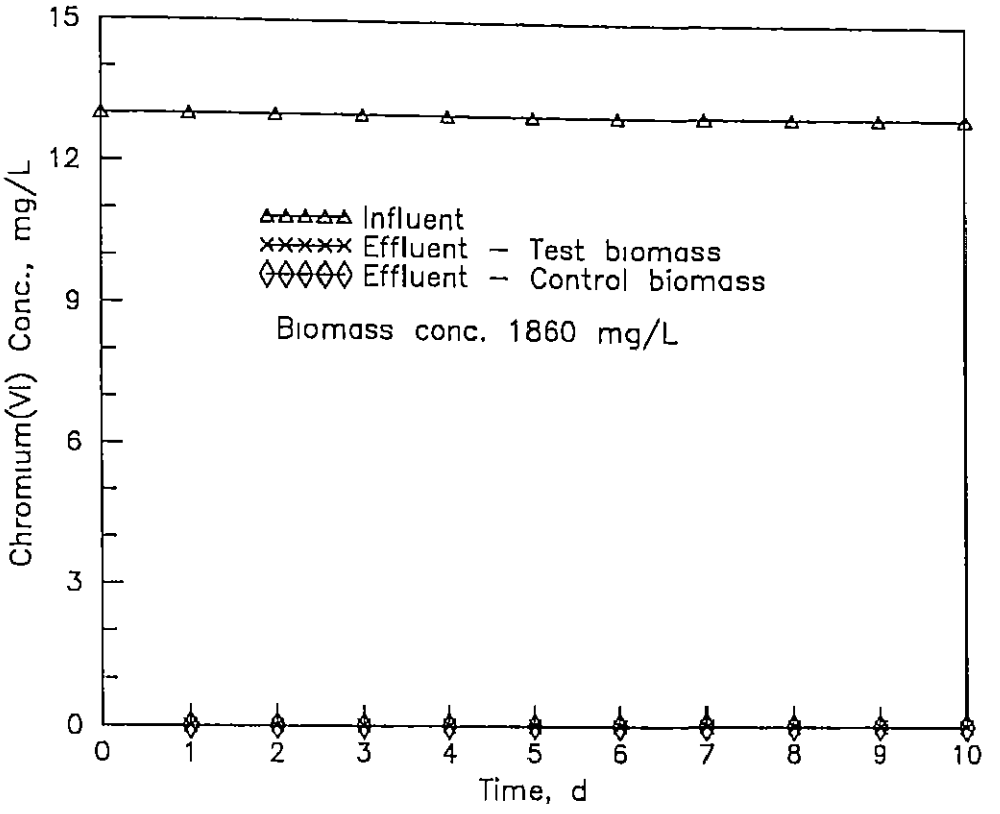


Fig. 5.8 Cr(VI) Reduction in Fed Batch System for Initial Cr(VI) Concentration of 13 mg/L

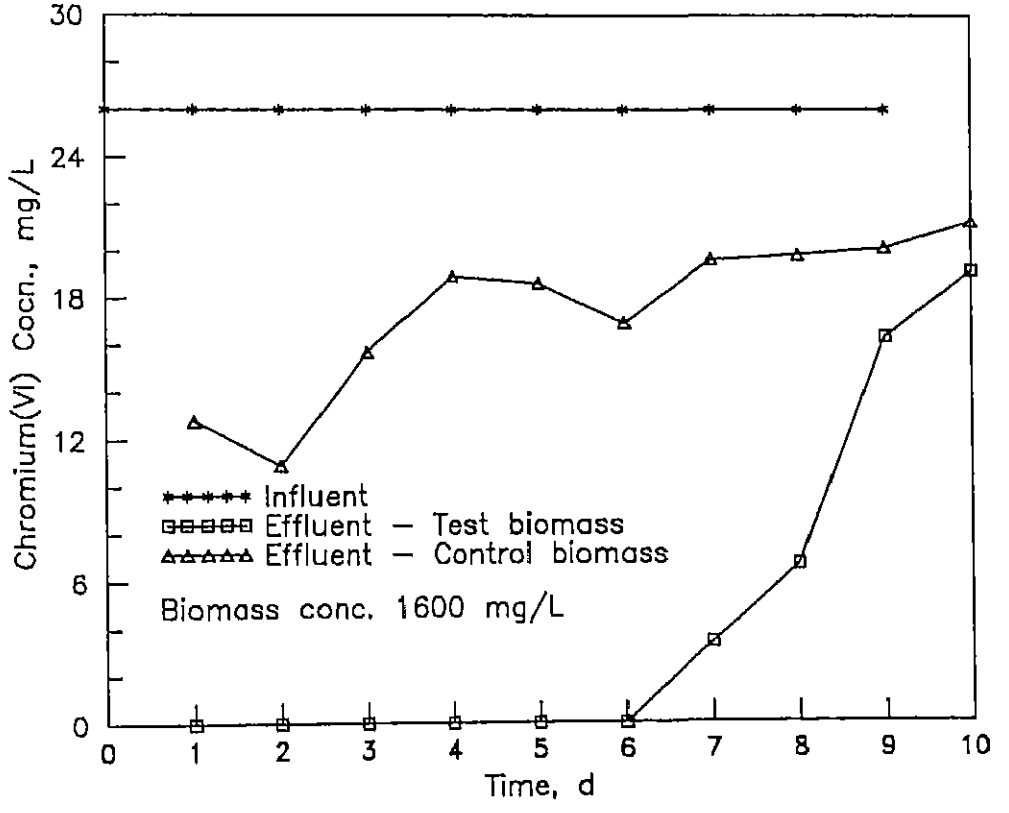


Fig. 5.9 Cr(VI) Reduction in Fed Batch System for Initial Cr(VI) Concentration of 26 mg/L

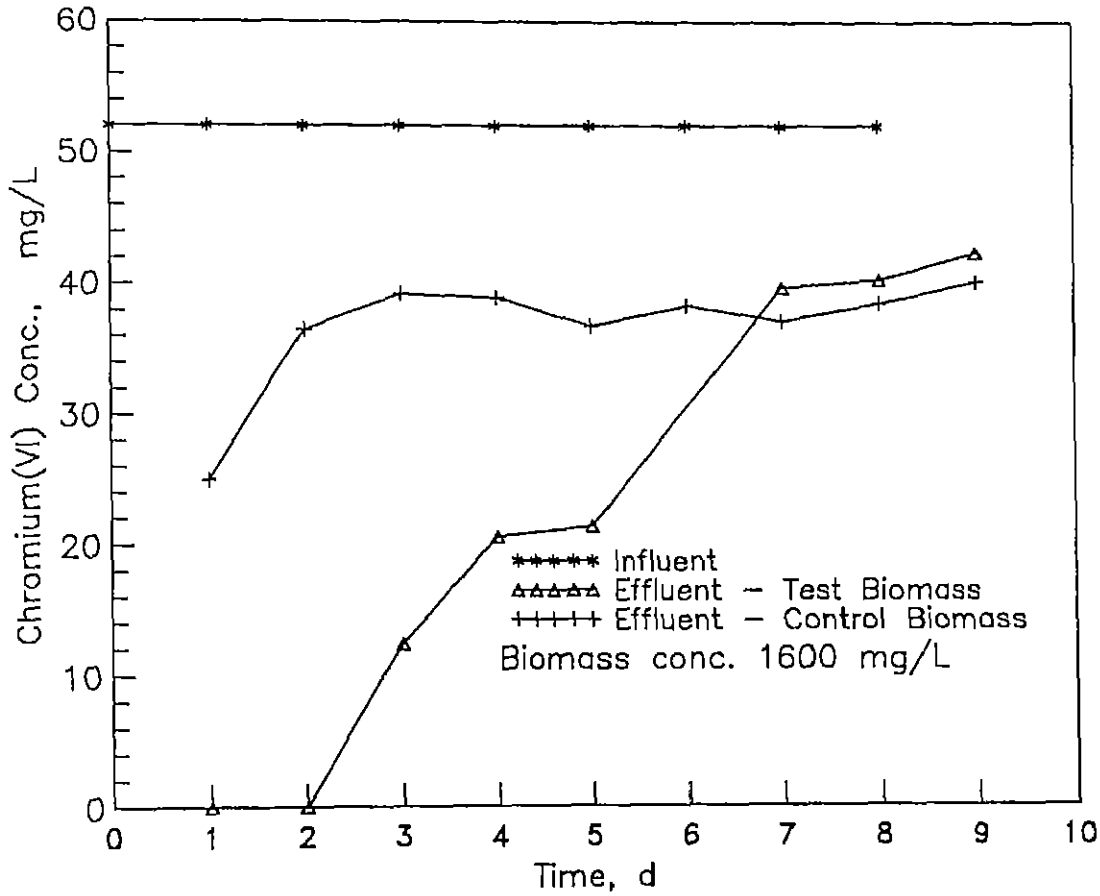


Fig. 5.10 Cr(VI) Reduction in Fed Batch System for Initial Cr(VI) Concentration of 52 mg/L

concentration, the test biomass could produce undetectable concentration of Cr(VI) in the effluent for 2 d beyond which there was chromium breakthrough.

The probable reason for this decrease appears to be loss of settling property of biomass as a result of exposure to higher Cr(VI) concentration and subsequent loss of biomass during the withdrawal of 900 mL of supernatant after 1 h settling time in once fed batch system. Besides this, the Cr(VI) toxicity as indicated in section 5.2 may also be responsible for the lower reduction rates.

Such observations were also made by by Gokay and Yetis (1991) who reported the presence of lesser biomass in an activated sludge system (with an BSRT above 8 h) when exposed to the chromium(VI) concentration of 25 mg/L. However, at 10 mg/L of Cr(VI) the performance was same as when the Cr(VI) concentration was negligible. Similar toxic effects of chromium was also observed by Imai and Gloyna (1990) who found that there was a considerable biomass loss even at Cr(VI) concentration of 5 mg/L.

5.4.2 Cr(VI) Reduction in Presence of Sewage as Electron Donor

The utility of sewage (M3 media)as electron donor in Cr(VI) reduction by two biomass was determined and the results are presented in Table 5.3 for an initial Cr(VI) concentration of 26 mg/L. It can be observed that there was 100% Cr(VI) reduction upto 2 d for test biomass after which it decreased while for the control biomass the Cr(VI) removal was very less. However, the

test biomass could effect 100% Cr(VI) reduction upto 6 d when malate (M2) was used as electron donor as per the results in previous section.

This may be result of the difference in the COD values of the carbon source added in M2 and M3 media as described in section 5.3.3. Another reason may be that malate being a intermediatory of Kerb's cycle produces NADPH (Nicotinamide Adinine Dinucleotide Phosphate) which reduces Cr(VI) more readily by donating electrons whereas bacteria may have to hydrolyse the sewage solids and the products of hydrolysis have to be glycolysed before producing the electron donors Ligy et al (1997) observed similar slower rate of reduction with pure culture of *B. coagulans* for glucose than for malate since glucose has to undergo bacterial glycolysis before yielding electron donors necessary for Cr(VI) reduction

Table. 5.3

Cr(VI) Reduction in Fed Batch System Using Sewage as Electron Donor

Day	Effluent Cr(VI) concentration, mg/L	
	Test Biomass	Control Biomass
1	ND	3.6
2	ND	14 6
3	8.4	16 4
4	12.3	16.7

Influent Cr(VI) concentration = 26 mg/L

Initial biomass concentration in reactors = 1454 mg/L

ND = Non-detectable

5.4.3 Performance of Fed Batch Reactors

As it was found that biomass could handle an initial Cr(VI) concentration of 13 mg/L very effectively, further experiments were conducted

After subjecting both biomass to a Cr(VI) concentration of 13 mg/L for 10 d, the initial Cr(VI) was increased to 26 mg/L to evaluate its response on Cr(VI) reduction The results are presented in Figure 5.11. The test biomass could easily handle the increased load of Cr(VI) and effected 100% Cr(VI) reduction upto study period of 18 d However, the control biomass could not produce the same results when the initial Cr(VI) concentration was increased to 26 mg/L. This shows that the test biomass has a better adaptability than control biomass.

The study with test biomass was continued further to obtain the failure point and the results are presented in Figure 5.12. The test biomass could handle the increased load of Cr(VI) (26 mg/L) effectively upto 18 d without posing any problem of settling. However, after 18 d of operation, settleability of Biomass was hampered but not Cr(VI) reduction. At this stage Cr(VI) feeding was discontinued to provide opportunity for the biomass for better settling However, upon resumption of feeding the Cr(VI) reduction had reduced and a steep breakthrough of Cr(VI) was obtained. Hence, it can be stated that upto 20 d the reactor can be operated with the test biomass after which augmentation of fresh biomass is required.

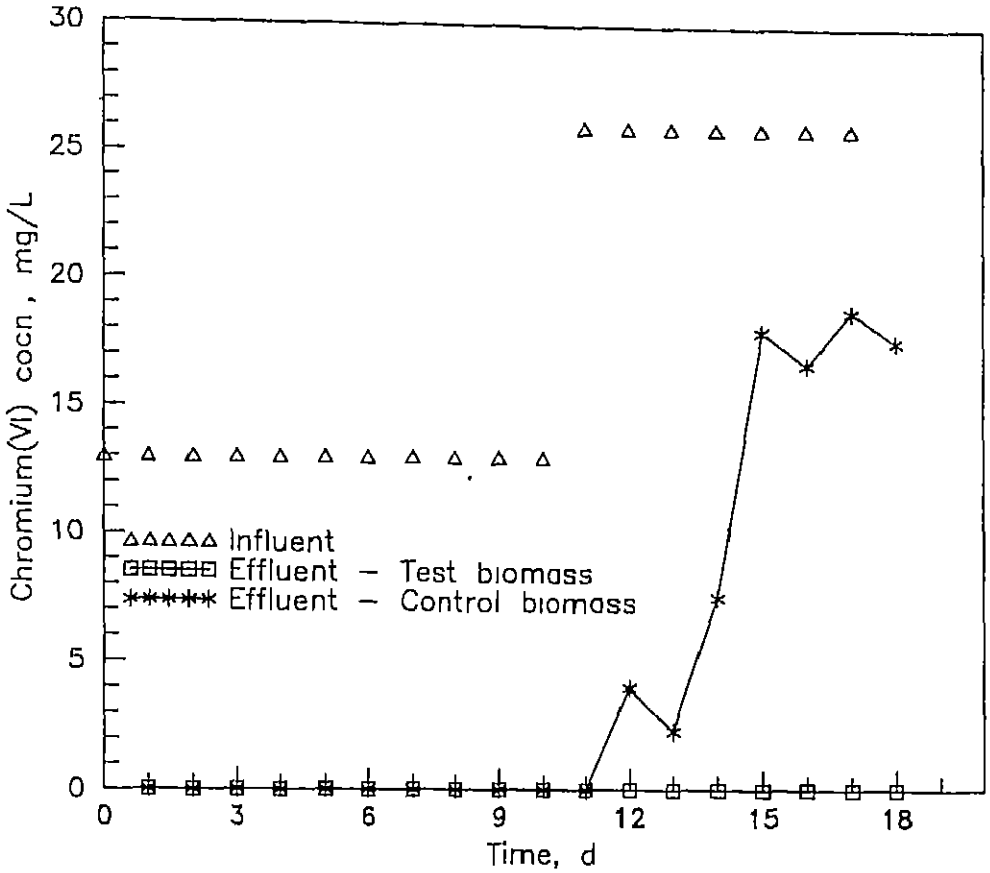


Fig. 5.11 Performance of Fed Batch System with Test and Control Biomass

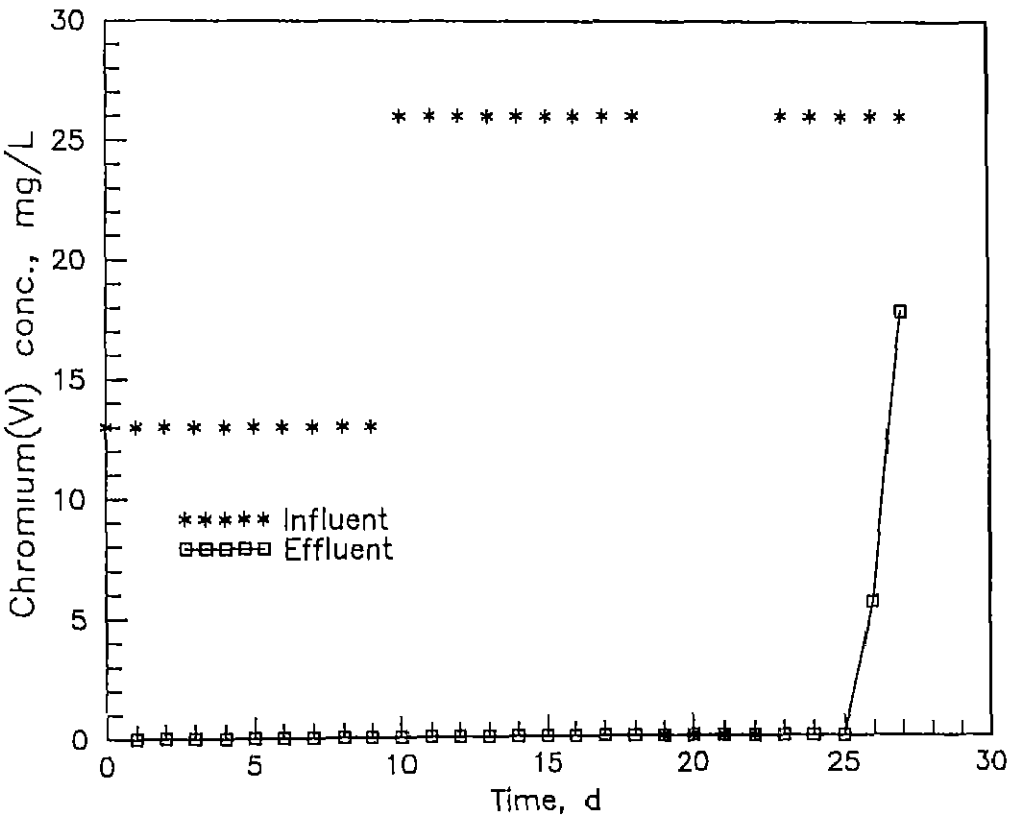


Fig. 5.12 Performance of Fed Batch System with Test Biomass

6. CONCLUSIONS

This study entails the potential of mixed culture biomass to reduce hexavalent chromium to trivalent form. Experiments conducted during the course of this study have revealed the following

1) Of the three bacterial isolates *B. coagulans* exhibited a higher specific Cr(VI) reduction capacity.
2) Increase in initial Cr(VI) concentration resulted in decrease of specific growth rate of *B. coagulans*.
3) Laboratory grown biomass on sewage supplemented with *B. coagulans* (test biomass) though reduced Cr(VI) at slower rate than pure culture of *B. coagulans* it could still be effective considering the economics of eliminating the sterilisation and costly solid-liquid separation process.
4) Test biomass showed a higher Cr(VI) reduction potential than control biomass which was grown in the absence of *B. coagulans*.
5) Domestic wastewater can be used as an inexpensive electron donor for biotransformation of Cr(VI) in place of costly malate. Use of domestic wastewater and malate with identical COD values resulted in similar specific reduction rates.
6) Reduction experiments in a fed batch reactor showed that both test and control biomass can produce effluents having non-detectable Cr(VI) concentration for an influent Cr(VI) of 13 mg/L. However, at 26 mg/L and 52 mg/L only test biomass could produce effluents having undetectable Cr(VI) concentration upto 6 and 2 days respectively.

7. SUGGESTIONS FOR FUTURE WORK

Based on this investigation following suggestions for future work may be made.

1) The effect of higher concentration of Cr(VI) on destruction of flocculant nature of biomass need to be investigated.
2) The mechanism of biotransformation of Cr(VI) needs to be delineated.
3) Studies on sequential batch reactors for handling toxic Cr(VI) wastes is to be undertaken.
4) The biosorption of Cr(III), the biotransformed product of Cr(VI) needs to be studied.
5) Studies on domestic wastewater of higher COD than employed in this study are required.
6) Biotransformation and biosorption studies using Cr(VI) bearing industrial wastewaters is to be studied

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